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http://dx.doi.org/10.1016/j.cub.2012.05.014

Axis Formation: Microtubules Push in the Right Direction

Live imaging reveals that the *Drosophila* oocyte nucleus is pushed by growing microtubules to break the radial symmetry of the oocyte and establish dorsoventral polarity.

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One of the attractions of studying dorsoventral axis formation in *Drosophila melanogaster* is the completeness with which the entire process has been analyzed starting from the first symmetry-breaking event in the ovary, up to the specification of particular cell types within the embryo [1,2]. There are not many cases in developmental biology where such a continuous causal chain can be constructed. In a recent paper, Daniel St. Johnston and colleagues [3] shed new light on the initial step of dorsoventral axis polarisation, the asymmetric positioning of the oocyte nucleus, which defines the dorsal side of the egg chamber and future embryo.

The importance of the oocyte nucleus' asymmetric position for dorsoventral axis formation in *Drosophila* has been recognized for a long time [4]. Even older, however, are similar observations for other insects: in the 1960s, for instance, Netzel [5] observed that oocyte nucleus migration in crickets breaks the rotational symmetry of the egg chamber and defines the plane of bilateral symmetry of the future embryo. Netzel also saw that follicle cells adjacent to the asymmetrically localized oocyte nucleus changed their morphology, and concluded that a signal emanating from the vicinity of the nucleus is received by the overlying follicle cells.

This idea has later been confirmed by studies of EGF signalling in *Drosophila* ovaries [6]. The mRNA of the TGFα-like ligand Gurken is concentrated within *Drosophila* oocytes close to the asymmetrically positioned oocyte nucleus. Gurken protein resulting from locally translated mRNA is secreted and activates the EGF receptor in the overlying follicle cells, thus providing spatial information to the follicle cells in relation to their distance from the oocyte nucleus. This patterning process results in the deposition of asymmetric cues in the eggshell, which is secreted by the follicular epithelium. These cues later orient formation of the dorsoventral axis in the embryo [7]. Recent comparative molecular and functional studies indicate that EGF signalling from the asymmetrically localized oocyte nucleus indeed represents an ancient mode of dorsoventral axis formation in insects [8]. However, the cell-biological mechanisms of asymmetric migration of the oocyte nucleus have, until recently, remained elusive.

In *Drosophila*, the early oocyte nucleus is located in a posterior position that is symmetric with respect to the short axis of the egg chamber (Figure 1). At mid-oogenesis, the nucleus migrates to the anterior, where it occupies an eccentric position along the perimeter of the anterior face of the oocyte, thereby breaking the radial symmetry of the egg chamber [9,10]. There is no indication that the final position of the oocyte nucleus is predetermined by any pre-existing asymmetry in the ovary, or by any external cues, such as gravity or the dorsoventral axis of the female abdomen. Thus, nuclear migration appears to be a genuine symmetry-breaking event [11].

In the past, two models have been suggested [2] for how the oocyte nucleus migrates. Both models incorporate the fact that the posterior follicle cells send a 'signal back' to the oocyte during mid-oogenesis, which initiates nuclear movement and the repolarisation of the microtubule network along the anterior-posterior axis [9,10]. In the first model, the oocyte nucleus is passive. Upon repolarisation of the network, the nucleus is then pulled to the anterior pole by dynein motors [12–17]. The other model is based on the observation that the nucleus migrates together with centrosomes and that the nucleus–centrosomal complex nucleates microtubules. Upon asymmetric nuclear positioning, the microtubules emanating from the nucleus were suggested to repolarize the microtubule cytoskeleton of the oocyte [18]. While the forces moving the nucleus were not specified in this model, one speculation was that microtubules growing from the nucleus–centrosomal complex could push the nucleus [2].

In the new study by Zhao, St. Johnston and colleagues [3], live imaging of oocyte nucleus migration, together with centrosome and microtubule dynamics, is used to rigorously test, for the first time, the different models of nuclear migration. In particular, the new results suggest that asymmetric nuclear positioning is neither dependent on, nor required for, the anterior-posterior polarisation of the oocyte. The main conclusions are based on the careful observation of the

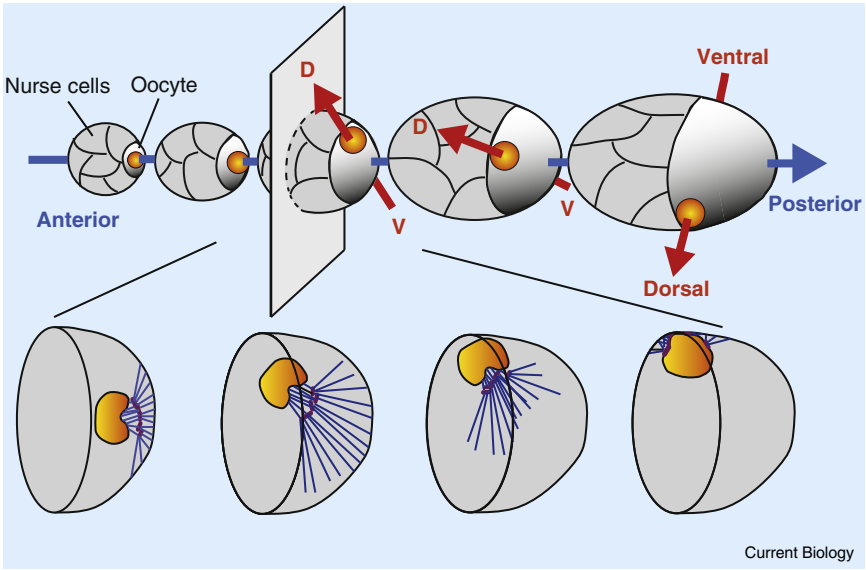

Figure 1. The polar axes of the *Drosophila* ovariole and the mechanism of nuclear migration.
Top: Within an ovariole, the egg chambers form a linear chain with young stages at the anterior and more mature stages at the posterior side. The follicular epithelium is not shown. The ovariole has a long anterior-posterior axis (blue arrow) and a perpendicular short axis indicated by a plane intersecting the third egg chamber. Radial symmetry is broken within this plane through the asymmetric positioning of the oocyte nucleus (orange), which defines the dorsoventral axis (red arrows, D: dorsal, V: ventral). Bottom: Successive stages of nuclear migration. The oocyte is depicted as a hemisphere. (Pink: centrosomes with associated MTOCs; blue: growing microtubules; orange: oocyte nucleus.)

shape of the oocyte nucleus. Shortly before and during migration, the nucleus is indented at the posterior. Treatment with a microtubule depolymerizing drug leads to the disappearance of this indentation, suggesting that it reflects the pushing forces of microtubules. These microtubules are nucleated from centrosomes located behind the nucleus (Figure 1). Strikingly, mutants with mispositioned centrosomes show ectopic nuclear indentations, while laser ablations of centrosomes locally abolish the indentations. By recording single microtubule plus-ends touching the nuclear membrane, Zhao *et al.* [3] show that approximately six microtubules are hitting the nucleus at any given time during migration. Using conservative estimates of the force produced by a single growing microtubule together with Stoke's law, which describes the movement of a sphere through a viscous medium, Zhao *et al.* [3] calculate that microtubule polymerisation can produce sufficient force to move the nucleus.

However, it is not the centrosomes themselves that are important for nuclear migration, as a mutant without centrosomes still exhibits normal nuclear behaviour [3]. The centrosomes rather serve as a marker for associated microtubule-organising centres (MTOCs), which nucleate the microtubules that push the nucleus towards the anterior. Thus, a prerequisite for successful migration during normal development is the positioning of MTOCs behind the nucleus, i.e. between nucleus and the posterior pole of the oocyte. This centrosome/MTOC positioning results from an early step of oocyte polarisation [19].

Another crucial observation in the work of Zhao *et al.* [3] is that indentations on the posterior face of the oocyte nucleus (indicating the presence of pushing forces) are seen well prior to any evidence of nuclear migration. This suggests that an anchoring force initially opposes the pushing force associated with the centrosomes. In mutants where the posterior polar follicle cells are not specified, and that therefore back signalling is lacking, the oocyte nucleus remains at the posterior, despite the persistence of the pushing force. This suggests that back signalling from the follicle cells is required to sever the anchor which would otherwise prevent nuclear migration. Interestingly, this severing function appears to be independent of the role the posterior signal plays in establishing anterior-posterior polarity of the microtubule cytoskeleton.

Simultaneous observation of changes in microtubule density and nuclear migration demonstrate that the nucleus may move anteriorly before the microtubule network is polarized, further showing that the oocyte nucleus is not passively following overall microtubule cytoskeletal polarity. This observation leaves at least the formal possibility that the anteriorly positioned nucleus is required for complete anterior-posterior polarisation of the oocyte. However, this too is unlikely, as certain *par-1* mutants lack microtubule anterior-posterior polarity, but still exhibit proper nuclear localization. Taken together, a picture emerges in which anterior-posterior and dorsoventral polarisation are largely independent processes.

Ensuring the orthogonal orientation of the body axes was the conceptual basis for the former proposal of a tight link between anterior-posterior and dorsoventral polarisation of the oocyte. The argument went as follows: the primary axis must be defined prior to the secondary axis, in order to establish an orthogonal relationship between the axes [9,10]. In the absence of any additional spatial information, simultaneous and independent formation of axes would lead to an unpredictable angular relationship between the two axes. However, this idea neglected the fact that the potential for two orthogonal axes is an inherent property of insect ovarian architecture (Figure 1).

The functional unit of all insect ovaries is the ovariole, a linear chain of egg chambers which progress in development from anterior to posterior [20] (Figure1). Thus, ovariole structure can be conceptualized as a tube with a long axis and an orthogonal short axis. The long axis gives rise to the anterior-posterior axis, whose symmetry is broken by the temporal order of development that is translated into anterior-posterior polarity of the egg chamber [19]. The short axis defines the plane of the future dorsoventral axis. Symmetry can be broken only within this plane.

Exactly this happens when the oocyte nucleus becomes localized on the outer perimeter of the anterior face

of the oocyte. This process is not necessarily dependent on particular mechanisms or paths by which it takes place, and Zhao *et al.* [3] indeed observed much variability. In addition, even if the MTOCs were mislocalized, as in the case of a *par-1* mutant, the nucleus would still end up in an asymmetric position. Altogether, it now appears that the perpendicular axes of the *Drosophila* oocyte are polarized independently of each other, and it is the geometric properties of the insect ovariole that ensure a perpendicular relationship between them.

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http://dx.doi.org/10.1016/j.cub.2012.05.038